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Bacteriophage cocktail supplementation to prevent *Escherichia coli* in native chickens in Tra Vinh province of Vietnam

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The bacteriophage cocktail (BC) was therapeutically used as an effective alternative to antibiotics for the control of colibacillosis in local chickens. The present study aimed to determine the alternative biocontrol effectiveness of BC against *Escherichia coli* (*E. coli*) in one-day-old broiler chicks in Tra Vinh province. Herein, the experiment design showed that BC was given at 10^9 CFU/mL, whereas *E. coli* was given at $10^{7,7}$ CFU/mL. A total of 120 chicks were randomly divided into three treatments in Experiment 1, including (i) negative control (NC) (gavaged with PBS); (ii) positive control (PC) (challenged with *E. coli*); and (iii) E1 (challenged with *E. coli* and gavaged with BC at 1, 3, 7, 14, and 21 days of age). In Experiment 2, 160 chicks were randomly assigned to one of four treatment groups consisting of (i) NC (gavaged with PBS); (ii) PC (challenged with *E. coli*); (iii) E2-1 (challenged with *E. coli* and gavaged with BC at 2 hours post-challenge, 3, 4, 5, 6, 7, 14, and 21 days of chick age); and (iv) E2-2 (challenged with *E. coli* and gavaged with antibiotic at the same time point as E2-1). According to the results of the present study, the mortality rate in the experiment groups using BC in both Experiments (E1 and E2-1) were significantly lower than those in the PC group. Besides, the results indicated that *E. coli* bacteria were only found in the heart, liver, and spleen of experimental chicks at 9 days of age in both Experiments. The bacterial density was lower than those in the PC group in both Experiments. Notably, there were no noticeable changes in BC and antibiotic groups. The findings of this study exhibited the promising effects of BC for controlling colibacillosis infections in native poultry.
Keywords: Bacteriophage, broiler chicken, colibacillosis, *Escherichia coli*, protection efficiency, treatment efficiency.

INTRODUCTION

The poultry industry plays a vital role in Vietnam's economy and supports millions of small-scale households (Bâtie et al., 2022). Vietnam contributed roughly 1,520 thousand tons of chicken meat to the global poultry market in 2021 (FAO, 2023). However, the extensive spread of poultry farming has also further increased disease emergence threats and economic costs (Nguyen et al., 2015; Nhung et al., 2017). Among the major diseases affecting broiler production in Vietnam, colibacillosis caused by pathogenic strains of Escherichia coli (E. coli) is one of the most prevalent and deadly among local broiler farms (Trung et al., 2016; Ergashevich & Salimovich, 2022; Kika et al., 2023). In the past, antibiotics were widely used as the first line of treatment for colibacillosis and were generally effective. Currently, the excessive and inappropriate use of antibiotics has led to a serious rise in antibiotic-resistant bacteria in poultry farming. The resistant strains can potentially be transmitted from animal products to humans; therefore, this is not only a problem for animal health, but it also raises major public health concerns (Nhung et al., 2015; Abreu et al., 2023). The identification of effective prevention and control measures against colibacillosis remains a paramount urgent matter for the world poultry sector. Bacteriophages have represented a new and promising control method for colibacillosis in chickens (Oliveira et al., 2010; Sorour et al., 2020; Eid et al., 2022; Alexyuk et al., 2023). The previous studies showed that bacteriophages could infect the host cells and multiply by taking over host cell synthesis, ultimately destroying the host and releasing new phages. Phage therapy is an effective method of treating drug-resistant bacterial infections in animals without altering the normal intestinal microflora. Indeed, previous studies reported that bacteriophage therapy is showing potential as an alternative biocontrol to prevent E. coli in native chickens in different geographical regions (Huff et al., 2006; Lau et al., 2010; Oliveira et al., 2010; Sorour et al., 2020; Eid et al., 2022; Alexyuk et al., 2023). Its application in the poultry industry gave a very targeted, environmentally friendly, and potentially sustainable method

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for the control of colibacillosis. As antibiotic resistance continues to rise, bacteriophage therapy emerges as a viable strategy to enhance disease control while reducing dependence on antibiotics in the poultry industry. However, studies on the effectiveness of bacteriophage in preventing E. coli in local chickens in Vietnam were limited (Ngu et al., 2020; Anh et al., 2022; Haider et al., 2023). A few studies paid attention to isolating and using a bacteriophage cocktail (BC) to control Salmonella in native broiler chickens in Vietnam (Ngu et al., 2022; Thu et al., 2023; Van et al., 2024). The present study therefore utilized the alternative biocontrol effect of BC against E. coli bacteria in native broiler chickens in Tra Vinh province of Vietnam. The findings of this study could contribute to expanding the basic knowledge on using BC as a therapeutic agent against colibacillosis and may support the control strategies of colibacillosis in the poultry industry in Vietnam in the near future.

MATERIALS AND METHODS

***Animal ethics*:** This study was carried out in accordance with the Animal Husbandry Law of Vietnam, number 32/2018/QH14. All the procedures of this experiment were performed under approval by guidelines from the Council for Ethics in Animal Experimentation, College of Agriculture, Can Tho University, number A10-02-2019/KNN.

***Isolation and preparation of bacteriophage*:** The bacteriophages were collected from environmental samples surrounding chicken households (water and soil) as well as from chicken (feces and intestine). Subsequently, 10g of the sample was put into a 50 mL falcon tube containing 20 mL of Tryptic Soy Broth (TSB) (Merck, Germany) medium and shaken at 150 rpm at 37℃ for 24 hours to culture the bacteriophage. Following, 2 mL of the mixture was centrifuged at 12,000 rpm for 10 minutes to remove sediments. The mixture was then transferred into an Eppendorf containing chloroform at 4℃ for 10 minutes. Finally, the mixture was centrifuged at 6,000 rpm for 5 minutes and collected the crude bacteriophage (Ngu et al., 2022). The E. coli serotype O1 was grown on Tryptone Soya Agar (TSA) (Merck, Germany) for 24 hours and prepared at a concentration of 10^8 CFU/mL. The bacteriophage solution with a concentration of 10^{-5} PFU/mL was utilized. Then, 0.1 mL of bacteriophage was mixed with 0.1 mL of bacterial suspension. 10-12 mL of melted TSB medium with 0.6% agar (previously kept in a thermostatic bath at 50°C for 30 minutes) was added, mixed well, and incubated at 37°C for 24 hours. After incubation, the bacteriophages were harvested and transferred into a sterile Eppendorf containing 0.9 mL of sterile distilled water, vortexed, and diluted to 10^{-5} PFU/mL. The whole experiment was replicated after every 24 hours for verification until the sizes and shapes of the plaque were uniform (Poxleitner et al., 2017). Choosing the representative bacteriophage after isolation and homogenization in size and shape. Isolation of the standard bacteriophage after homogenization in size and shape. The bacteriophage was harvested and transferred into a sterile Eppendorf containing 1.5 mL of TSB medium. Thereafter, 0.1 mL of bacterial suspension with a concentration of 10^8 CFU/mL was transferred into the TSB medium and incubated at 37°C for 24 hours. Then, 0.1 mL of chloroform solution was added into the Eppendorf, vortexed, and incubated for 2 hours. The Eppendorf was then centrifuged at 6,000 rpm at 4°C for 15 minutes to rule out sediments. pH tolerance in the supernatant was also checked (Verma et al., 2009). Lastly, 1 mL of clear supernatant was pipetted into a sterile Eppendorf and stored in the dark at 4°C (Phuong et al., 2022). Bacteriophages were screened based on the results in host range, pH range, and lytic ability against E. coli (Anh et al., 2022). In the present study, the BC (L1R13, L2N04, and L2R04) were selected and utilized for further experiments (Figure 1).

Figure 1. Bacteriophage L2R04 was used in the present study.

***Animals and diets*:** A total of 388 one-day-old native broiler chicks were used in the current study for two experiments. The vaccine program was Marek disease at 1 day of age, Newcastle disease at 1 and 21 days of age, Gumboro disease at 7 days of age, Fowlpox disease at 10 days of age, and H5N1 at 14 days of age. The chicken house was divided into several pens and considered as replicates. Each pen was 1 m^2 and separated by silver partitions. All chicks raised in this experiment were reared under the same conditions. They were fed twice a day, once at 7.00 am and once at 2.00 pm, with the same amount of feed in each experimental pen, using commercial feed (Gold Coin company, Vietnam) without antibiotics as a basal diet. The native broiler chicks were given free access to drinking water.

Experimental design

***Determination of Lethal dose (LD) 50*:** A completely randomized design was selected, 108 one-day-old native broiler chicks were randomly divided into six treatments (6 chicks per treatment × 3 replicates × 6 treatments = 108 chicks) consisting of a negative control (NC) group and five experimental groups (L1, L2, L3, L4, and L5). Chicks in the NC group were gavaged with 1 mL of Phosphate-buffered saline (PBS) solution. Meanwhile, chicks in the L1, L2, L3, L4, and L5 groups were challenged with 1 mL of *E. coli* bacteria at a concentration from 10^5 to 10^9 CFU/mL, respectively. All experimental chicks were monitored for five days, with mortality, symptoms, and lesions recorded. Necropsies were performed on dead chicks, and internal organs (heart, liver, and spleen) were collected to culture *E. coli* bacteria for confirmation. The LD50 was determined using the Reed & Muench (1938) formula.

***Experimental design 1 to evaluate the protective effect of BC against E. coli in chicks*:** The experiment followed a completely randomized design with a total of 120 chicks (10 chicks per treatment × 3 treatments × 4 replicates). The treatments were: (i) the negative control (NC) group: Chicks were gavaged with 0.1 mL of PBS at 2 days of age; (ii) the positive control (PC) group: Chicks were challenged with 1 mL of *E. coli* bacteria once at an LD50 dose at 2 days of age; and (iii) E1 group: Chicks were challenged with 1 mL of *E. coli* bacteria once at an LD50 dose at 2 days of age and were gavaged with BC at a dose of 1 mL containing 10^9 CFU/mL at 1, 3, 7, 14, and 21 days of age. All chickens were monitored for 23 days, with mortality, symptoms, and lesions recorded. Necropsy was randomly performed on one chick per replicate at 9, 16, and 23 days of age, totaling 36 chicks (1 chick × 4 replicates × 3 treatments × 3 times). After necropsy, samples (heart, liver, and spleen) were collected to determine bacterial density.

***Experimental design 2 to evaluate the therapeutical effect of BC against E. coli in chicks*:** The experiment followed a completely randomized design with a total of 160 chicks (10 chicks per treatment × 4 treatments × 4 replicates). The treatments were: (i) the negative control (NC) group: Chicks were gavaged with 0.1 mL of PBS at 2 days of age; (ii) the positive control (PC) group: Chicks were challenged with 1 mL of *E. coli* bacteria once at an LD50 dose at 2 days of age; (iii) E2-1 group: Chicks were challenged with 1 mL of *E. coli* bacteria once at an LD50 dose at 2 days of age and were gavaged with BC at a dose of 1 mL containing 10^9 CFU/mL at 2 hours after infection, 3, 4, 5, 6, 7, 14, and 21 days of age; and (iv) E2-2 group: Chicks were challenged with 1 mL of *E. coli* bacteria once at an LD50 dose at 2 days of age and were gavaged with gentamicin at a concentration of 1 g/4 L water at 2 hours after infection, 3, 4, 5, 6, 7, 14, and 21 days of age. All chickens were monitored for 23 days, with mortality, symptoms, and lesions recorded. Necropsy was randomly performed on one chick per replicate at 9, 16, and 23 days of age, totaling 48 chicks (1 chick × 4 replicates × 4 treatments × 3 times). After necropsy, samples (heart, liver, and spleen) were collected to determine bacterial density.

***Sampling and confirmation of E. coli*:** The samples were collected from chicks, and then the bacteria were cultured on Tryptone Bile X-glucuronide (TBX) medium according to the Vietnamese national standards (TCVN 5155-1990). For confirmation of *E. coli* bacteria in dead chicks, four samples with characteristic lesions of colibacillosis were collected from chicks in each experiment (two chicks per experiment) to isolate *E. coli* bacteria. PCR was performed using primer sequences and thermal cycling conditions according to the previous study (Wang et al., 2014). The primer pair were ECO1-F (CGATGTTGAGCGCAAGGTTG) and ECO1-R (CATTAGGTGTCTCTGGCACG), producing a 263 bp amplicon. Following, positive samples were sent to a commercial company (Can Tho, Vietnam) for sequencing using the Sanger method. The obtained sequences were manually checked via BioEdit package software version 7.2.5 and compared with reference strains available in GenBank. Nucleotide and amino acid identities were carried out using BioEdit package software version 7.2.5, and the heatmap was performed to display the nucleotide and amino acid identities using TBtools-II package software (Chen et al., 2023).

***Statistical analysis*:** Primary data were processed using Microsoft Excel package software version 2016 and statistically analyzed using the ANOVA test in Minitab package software version (State College, PA, USA). A p-value of less than 0.05 was considered statistically significant among treatments.

RESULTS

***BC isolation*:** A total of 39 samples including 5 water samples, 3 fecal samples, 6 soil samples, and 25 intestinal samples were collected from chicken households to isolate the bacteriophages. The results indicated 11 (28.21%) bacteriophages were isolated from 39 samples. Specifically, 3 bacteriophages were isolated from 5 water samples (60.0%), 2 bacteriophages were obtained from 6 soil samples (33.33%), and 6 bacteriophages were isolated from 25 intestinal samples (24.0%). No bacteriophages were recovered from the 3 fecal samples (0%) (Figure 2).

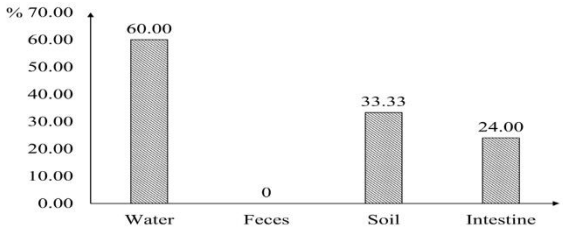


Figure 2. Bacteriophage isolation results in the current study.

***pH tolerance*:** The current result revealed that the bacteriophage viability varied under different pH conditions. Specifically, the viability of bacteriophage increased continuously from acidic to neutral (pH 2 to pH 7). A total of 7/11 (63.64%) samples survived at pH 2 and pH 3. At pH 4, pH 5, and pH 6, 8 (72.73%) samples survived, meanwhile, all 11 samples (100%) survived at pH 7. Based on these findings, the BC of L1R13, L2N04, and L2R04 from intestinal and water samples were selected for further investigations to determine their feasibility for the protective and therapeutical effects of BC against *E. coli* O1 bacteria in broiler chicks (Table 1).

Table 1. Evaluation of pH tolerance of bacteriophages against *E. coil*.

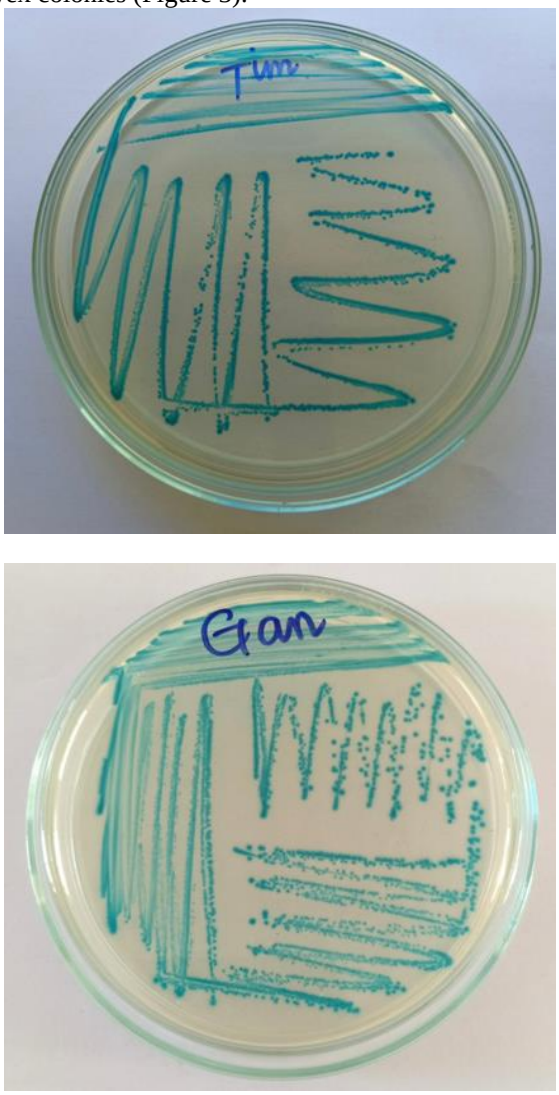
No.	Sample	pH level					
		2	3	4	5	6	7
1	L1N01	-	-	-	-	-	+
2	L1R01	+	+	+	+	+	+
3	L1R04	-	-	+	+	+	+
4	L1R13	+	+	+	+	+	+
5	L2D02	-	-	-	-	-	+
6	L2D03	+	+	+	+	+	+
7	L2N01	-	-	-	-	-	+
8	L2N04	+	+	+	+	+	+
9	L2R01	+	+	+	+	+	+
10	L2R02	+	+	+	+	+	+
11	L2R04	+	+	+	+	+	+
Bacteriophage (+)		7	7	8	8	8	11
Percentage (%)		63.64	63.64	72.73	72.73	72.73	100

***Determination of LD50 dose*:** In the present study, the LD50 was determined using the formula of Reed & Muench (1938). The mortality rate of chicks increased with higher bacterial concentrations. The lowest mortality rate (0%) was observed at concentrations of 10^5, 10^6, and in the NC group. The highest mortality rate occurred in the L5 group (10^9 CFU/mL), where 61.11% of the chicks died (Table 2). According to the formula, the LD50 used for experimental chicks was $10^{7,7}$ CFU/mL.

Table 2. Mortality rate of chicks in the LD50 experiment.

Treat.	No. of chicks	Conc. (CFU/mL)	No. of death (chick)	Percentage (%)
NC	18	PBS	0	0
L1	18	10^5	0	0
L2	18	10^6	0	0
L3	18	10^7	7	38.89
L4	18	10^8	10	55.56
L5	18	10^9	11	61.11

During the experiment, typical symptoms and lesions were observed in experimental chicks including diarrhea, lethargy, ruffled feathers, dry legs, swollen and hemorrhagic intestines, a swollen spleen, a gas-filled cecum, pericardial effusion, and a swollen, fibrinous liver. Additionally, the bacteria were cultured to confirm the presence of *E. coli* infection, samples were collected from the heart, liver, and spleen of chicks and cultured on TBX. The culture results showed the presence of *E. coli*, characterized by typical green, round, and slightly convex colonies (Figure 3).



(A)

(B)

Figure 3. Typical colony of *E. coli* on TBX medium. (A) The sample was collected from the heart and (B) the liver of the experimental chicks.

The protective effect of BC against *E. coli* in chicks in Experiment 1

***Mortality rate of chicks in Experiment 1*:** The mortality rate of chicks in the E1 group was significantly lower compared to the PC group ($p = 0.01$). In the NC group, chicks were gavaged with PBS solution, and no dead chicks were observed in this group (Figure 4).

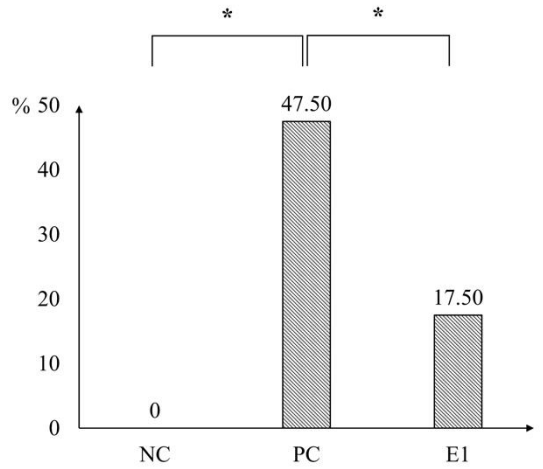


Figure 4. Mortality rate of chicks in Experiment 1.
** The significant difference among groups (p < 0.05) using the ANOVA test in Minitab software*

During the experiment, chicks exhibited typical symptoms of colibacillosis, including diarrhea, fatigue, lethargy, ruffled feathers, and dry legs. Additionally, necropsy revealed typical lesions associated with *E. coli* infection such as inflamed and swollen liver, pericardial effusion, as well as enlarged and hemorrhagic intestines. Furthermore, chicks in the PC group developed diarrhea within three days after infection, and symptoms of respiratory failure were also observed in this group.

***Bacterial density in Experiment 1*:** The results showed that *E. coli* bacteria were found in the heart, liver, and spleen of experimental chicks at 9 days of age, however, they were completely absent in the NC group. The PC group was the highest bacterial density (4.64-5.28 $\log_{10}$ CFU/mL) compared with other groups (p = 0.01). At 16 and 23 days of age, no pathogenic bacteria were detected in any groups (Figure 5).

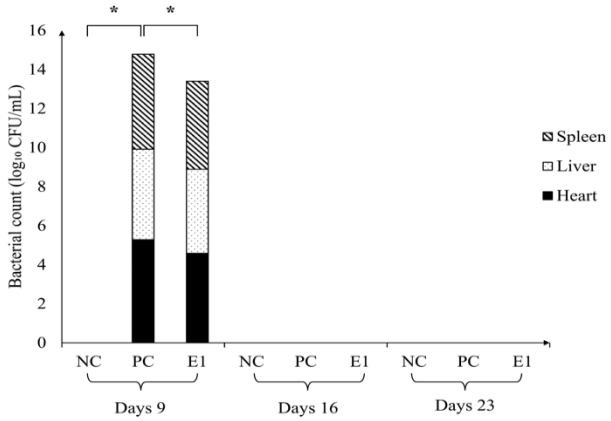


Figure 5. *E. coli* bacterial density in Experiment 1.
** The significant difference among groups (p < 0.05) using the ANOVA test in Minitab software*

The therapeutic effect of BC against *E. coli* in chicks in Experiment 2

***Mortality rate of chicks in Experiment 2*:** Herein, the lowest mortality rate was observed in the NC group, meanwhile the highest was in the PC group. The mortality rate in the experiment group using BC (E2-1) was significantly lower compared to the PC group. The difference was statistically significant (p = 0.01). Besides, the mortality rate in the E2-2 group was lower than in the PC group (p = 0.01). However, there was no statistically significant (p > 0.05) between E2-1 and E2-2 groups (Figure 6). The symptoms and lesions of experimental chicks were the same as in Experiment 1.

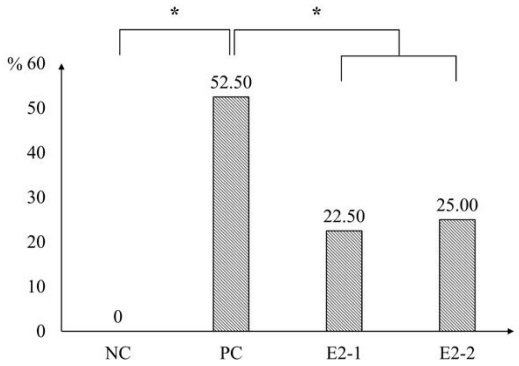


Figure 6. Mortality rate of chicks in Experiment 2.
** The significant difference among groups (p < 0.05) using the ANOVA test in Minitab software*

***Bacterial density in Experiment 2*:** The results of the current study indicated that bacteria were found in chicks at 9 days of age, however, they were not detected in chicks at 16 and 23 days of age. Moreover, *E. coli* bacteria were demonstrated in heart, liver, and spleen samples. The bacterial density in the PC group was the highest (4.75-5.15 $\log_{10}$ CFU/mL) compared to other groups (p = 0.01). The mortality rate of experimental chicks was not statistically significant (p > 0.05) between E2-1 (4.49-4.56 $\log_{10}$ CFU/mL) and E2-2 (4.48-4.55 $\log_{10}$ CFU/mL) groups. In contrast, the lowest mortality rate was found in the NC group (Figure 7).

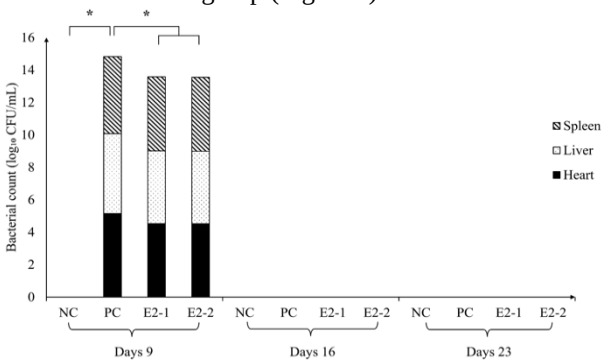


Figure 7. *E. coli* bacterial density in Experiment 2.
** The significant difference among groups (p < 0.05) using the ANOVA test in Minitab software*

Identification of E. coli: The samples were collected from experimental chicks, and bacteria were isolated and cultured for identification. PCR technique was carried out to amplify the primer sets at 263 bp in length. In this study, two represented samples in individual experiments were chosen for sequencing. After sequencing, the obtained sequences were compared with the reference strains in the NCBI database. The results indicated the high homology of nucleotide and amino acid identities between the field strain and the reference strains available in GenBank, accounting for 98.1-100 and 98.0-100, respectively (Figure 8).

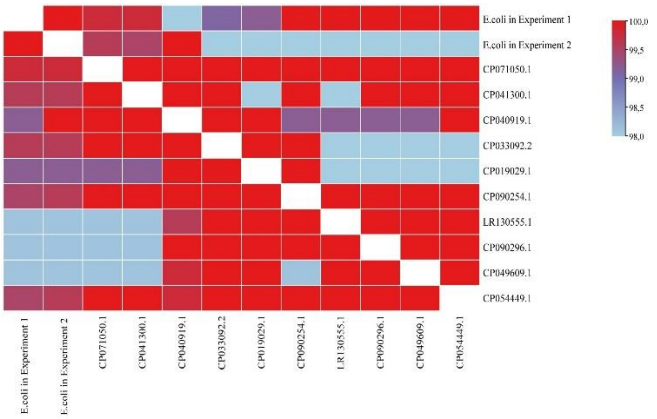


Figure 8. The nucleotide identity (below the diagonal) and amino acid identity (above the diagonal) of field *E. coli* bacteria in the present study and the reference strains on GenBank.

DISCUSSION

To date, bacteriophage is publicly accepted as one of the promised therapies to prevent colibacillosis in the poultry industry worldwide (Huff et al., 2006; Lau et al., 2010; Oliveira et al., 2010; Sorour et al., 2020; Eid et al., 2022; Alexyuk et al., 2023). However, studies on using bacteriophage isolated in local locations as a therapeutic agent against chicken colibacillosis in the native broiler chicken of Vietnam seem to be rare (Ngu et al., 2020; Anh et al., 2022). Therefore, the present study used BC to prevent colibacillosis in one-day-old native broiler chicks in Tra Vinh province of Vietnam. The results of the current study were first demonstrated that supplementing native broiler chicks with BC may reduce the mortality rate and bacterial density in internal organs.

Colibacillosis is a fatal infectious disease and is one of the most popular diseases in the poultry industry globally. It is caused by *E. coli* bacteria, which is mostly detected in the gastrointestinal tract and mucosal surfaces of the host (Lutful Kabir, 2010; Ergashevich & Salimovich, 2022; Kika et al., 2023). Avian Pathogenic E. coli (APEC) is a serious pathogen in the poultry industry that causes several localized or systemic diseases in chickens (Ergashevich & Salimovich, 2022; Kika et al., 2023). There were numerous serotypes of APEC circulating worldwide, however, three main serotypes of APEC (O1, O2, and O78) were responsible for over 80% of outbreaks (Ghunaim et al., 2014). Thus, the present study utilized E. coli bacteria serotype O1 as the agent for experimental broiler chicks. The results of the LD50 Experiment demonstrated that all the challenged chicks displayed the typical symptoms and lessons of colibacillosis similar to the results of the prior study (Ergashevich & Salimovich, 2022). This result implied that *E. coli* bacteria serotype O1 was the pathogenic strain for the experimental chicks in the current study. Furthermore, re-isolation results additionally indicated that *E. coli* was the main agent that caused and was lethal to experimental chicks. Besides, the nucleotide and amino acid identity results demonstrated that there was high homogenization between the field *E. coli* strains and the reference strains on GenBank.

In Experiment 1, the experimental chicks were gavaged with BC 1 day before being challenged with *E. coli* bacteria at an LD50 dose and frequently gavaged with BC after challenge (3, 7, 14, and 21 days of age). The results revealed that using BC tended to decrease the mortality rate of chicks compared to the PC group. Moreover, the experimental chicks exhibited the typical symptoms and lessons of colibacillosis, meanwhile, no symptoms and lessons were observed in chicks in the NC group. The reduction in the mortality rate of experimental chicks demonstrated the potential ability to prevent *E. coli* bacteria in local broiler chicks of BC. The current result was similar to the previous study, the lower mortality rate was observed in the bacteriophage group (10%) compared to the PC group (30%) (Sorour et al., 2020). According to the study of Anh et al. (2022), BC isolated from the local environment significantly reduced the mortality rate in experimental groups in comparison with the PC group as well as also improved growth performance and carcass in local broiler chickens. Besides, the bacterial density significantly decreased in the BC group compared to the PC group. The results of the current study were similar to those of Anh et al. (2022), during the experiment, *E. coli* was not found in the heart, liver, and spleen of the experimental chicks in the unchallenged group. In addition, the bacterial density decreased on day 7 after administration with BC and was eliminated after day 49 of the treatment. A study by Sorour et al. (2020) also showed that the most effective day of BC was reported to be day 23 of the experiment, a great reduction of E. coli O119 in the cecum, heart, and liver of the chicks. Additionally, another study found a high presence of E. coli in the heart, liver, and spleen of the challenged chicks following 5 days. However, the density of *E. coli* decreased in the heart, liver, and spleen of the experimental chicks in the BC group (Naghizadeh et al., 2019; Enriquez et al., 2024). This indicated that BC suppresses the growth of *E. coli* in the internal organs of chicks. The reduction of bacteria in the internal organs contributed to the increased survival rate of

chicks when challenged with pathogenic bacteria. The findings of this study imply that BC exhibited a potential effect on the protection of *E. coli* bacteria in local broiler chicks.

During Experiment 2, experimental chicks were challenged with *E. coli* bacteria at LD50 dose, and gavaged with BC at 2 hours post-challenge, the chicks were subsequently gavaged regularly with BC at 3, 7, 14, and 21 days of age. The results showed that there was a decrease in the mortality rate of chicks in the experimental groups compared to the PC group. However, no statistically significant variation was noted between antibiotic (E2-1) and BC groups of chicks in mortality. Furthermore, the normal symptoms and lessons of colibacillosis were noted in the PC as well as the test groups. On the other hand, there were no symptoms and lessons noted in chicks in the NC group. The negligible mortality rate certified that BC was effective in inhibiting *E. coli* bacteria and reduce mortality in infected chicks. Previous research by Oliveira et al. (2010) had shown the effectiveness of bacteriophages as a therapy for chicken inoculated with *E. coli*, mortality rate reduced over the weeks. In all groups, after three weeks of treatment, all groups effectively controlled *E. coli* infection, with mortality rates in all treatment groups being less than 1%. Additionally, Anh et al. (2022) reported that no mortality was observed in the NC group and the BC group. The highest mortality rate was observed in the PC group. The BC could affect specific bacterial species, and combining multiple bacteriophages could increase bacterial penetration and eradication. Furthermore, Wernicki et al. (2017) bacteriophages as known as prokaryotes widely distributed in nature, with life cycles closely linked to bacterial cells. Bacteriophages were bacterial parasites due to the lack of cellular structures and necessary enzymes for food absorption, protein synthesis, or independent replication, meaning they can only reproduce within a living cell. Recent research indicated that the use of bacteriophages was safe for experimental chickens, with minimal side effects due to the highly specific of bacteriophages to individual bacterial strains and they were a minor impact on the natural bacterial flora of the hosts (Sulakvelidze et al., 2001). Besides the antibacterial effects, bacteriophages may additionally interact with the host immune system in complex pathways. The previous studies suggested that bacteriophages could modulate immune responses, either by influencing innate immunity or by interacting with host cells. Bacteriophages stimulated the production of anti-inflammatory cytokines, potentially contributing to a balanced immune response. Conversely, bacteriophages could be recognized by the immune system of the host, leading to the production of neutralizing antibodies that may limit their long-term efficacy (Gorski et al., 2003; Popescu et al., 2021). Despite these interactions, phage therapy was found to be low in immunogenicity in the majority of instances and, therefore, a promising and well-tolerated alternative to antibiotics in broiler production. Similar to Experiment 1, bacterial density was significantly lower in the BC group compared to the PC group. The results of bacterial density were consistent with the findings of Lau et al. (2010), *E. coli* population was reduced in the liver, heart, and spleen of the experimental chicks, and was eliminated from the blood of chicks in the BC group. Similarly, Kaikabo et al. (2017) reported that bacterial load was significantly reduced in the feces of Cobb 500 breed, from 2.31×10^8 CFU/mL to 1.95×10^4 CFU/mL, after three weeks of oral treatment with BC. Surprisingly, there was no significant difference in the mortality rate and bacterial density between the BC group and the antibiotic group. These findings imply that BC was indeed able to suppress the growth of the pathogen in local broiler chicks.

It is also well established that bacteriophages have been very selective and targeted specific pathogenic bacterial populations without harming the host gut microbiota to a large degree. Therefore, bacteriophages can reduce the risk of gut dysbiosis and provide balanced microbial environment of the host. This is a unique feature of bacteriophages compared to broad-spectrum antibiotics (Lekunberri et al., 2017). Furthermore, long-term use of bacteriophage therapy would significantly reduce the dependence of poultry breeding on antibiotics, eventually fight antibiotic resistance, and minimize antibiotic residues in food (Abd-El Wahab et al., 2023). Moreover, bacteriophages broke down spontaneously after completing their bacterial lysis cycle compared to environmental survival and development of antibiotic resistance by antibiotics. Hence, the long-term ecological impact of using bacteriophages is mostly reported to be low in relation to antibiotics (Koskella et al., 2022). However, the long-term effects of bacteriophage require further investigations, particularly regarding bacterial resistance, environmental effects, and large-scale application strategies. Follow-up studies should be encouraged to optimize bacteriophage effectiveness and ensure its safety as a therapy for poultry farming. Herein, the unavoidable limitations were displayed in the current study. First, only native broiler chicks from 1 to 21 days old were utilized in both Experiments. Further investigations should be performed on a wide range of ages and breeds of experimental chickens to clearly indicate the effects of BC in chickens. Second, the present study was carried out in the small-scale household model, therefore, the applicability of our results was limited. Investigation of the effect of BC in other farming systems should be encouraged by additional studies. Long-term effects of BC were excluded in the current study. Thus, further examinations should be promoted to explore the long-term effects of BC in poultry farming.

***Conclusion*:** In sum, the findings of the current study indicated that using BC exhibited a potential effect on the

protection as well as treatment of *E. coli* bacteria in local broiler chicks in Tra Vinh province of Vietnam.

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